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Size, Hemoglobin Content, and Acid-Soluble Phosphorus of Erythrocytes of Rabbits with Phenylhydrazine-Induced Reticulocytosis.

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Systematic studies of the distribution of acid-soluble phosphorus compounds in the blood cells of various animal species have been under way for some time in our laboratories.[1,2] Present evidence suggests that the distribution may vary with the stage of maturation of the erythrocytes. Kay[3] reported observations on the distribution of acid-soluble P in the bloods of patients with splenic anemia, acholuric jaundice, hemolytic icterus during crises, and pernicious anemia during treatment with liver extract. Such bloods, which are characterized by increased percentages of reticulocytes, exhibited relatively high cellular concentrations of organic acid-soluble P. There was also indirect evidence that nucleoprotein, generally believed to be absent in mature erythrocytes, was present in these bloods in amounts roughly proportional to the reticulocyte count.

In order to elucidate the suggested relationship further we have now determined the distribution of acid-soluble P in the bloods of rabbits following repeated injections of phenylhydrazine which, as reported by Watson,[4] leads to the appearance of large numbers of reticulocytes in the blood. In the bloods of the rabbits so treated, estimations were made of the inorganic P, total acid-soluble P, adenosine triphosphate P, and in some instances the diphosphoglycerate as well as of the dimensions and of the hemoglobin content of the cells. Measurements of the osmotic fragility of the cells, which were made at the same time, will be described in a later communication.

Methods. The methods used for the collection of blood and for the determination of the various phosphorus fractions have been described previously.[1] Reticulocyte counts were made on wet preparations. A drop of blood was spread on a slide under a coverslip on which a film of brilliant-cresyl-blue had been dried. The number of reticulocytes seen during the counting of 500 erythrocytes was noted; if the percentage of reticulocytes was low, 1000 erythrocytes were counted. Two pipettes and 2 hemocytometers, certified by the Bureau of Standards, were used. Ten large squares in each of the chambers—a total of 40 large squares (0.16 mm^3)—were counted.

The volume of packed cells was determined by the method of Guest and Siler.[5] The hemoglobin was measured in an Evelyn colorimeter, using filter 540.

Experimental. Full grown white New Zealand rabbits weighing 2.5 to 3.5 kg were used. To produce reticulocytosis a 3.3% solution of phenylhydrazine hydrochloride, adjusted to pH 7 by the addition of NaOH, was administered subcutaneously. As a rule the phenylhydrazine was injected according to the following schedule: first day, 33 mg; second day, 66 mg; fourth day, 33 mg; and from then on, 33 mg every second day. The total dose given and the intervals between the injections were varied in individual animals according to the degree of anemia that developed and the losses of weight observed from day to day. Usually, in the successful experiments, the animals lost little weight. Among the rabbits so treated the mortality was fairly high during the first 4 days, but few of the animals surviving longer periods of time died. Blood samples for chemical analyses were drawn before the first injection and at varying intervals during the

1 Guest, G. M., and Rapoport, S., *Physiol. Rev.*, 1941, **21**, 410.

2 Rapoport, S., and Guest, G. M., *J. Biol. Chem.*, 1941, **138**, 269.

3 Kay, H. D., *Brit. J. Exp. Path.*, 1930, **11**, 148.

4 Watson, C. J., and Clarke, W. O., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 65.

5 Guest, G. M., and Siler, V. E., *J. Lab. and Clin. Med.*, 1934, **19**, 757.

TABLE I.

Size, Hemoglobin Content, and Acid-Soluble P in Erythrocytes of Rabbits Treated with Phenylhydrazine.

Animal No.	Vol. packed cells, %	Rbc count, mill.	Mean cell vol., μ^3	Hb conc. cells, g/100 cc	Mean Hb per cell, γγ	Reticulocytes		ATP cells, mg/100 cc	OASP cells, mg/100 cc	Notes
						Total, %	Heavy, %			
C 338	41.1	6.34	65	33.8	21.9	2	0	14.1	73.6	Before treatment
	17.7	2.45	72	29.0	21.0	88	27	18.7	79.6	4th day "
	32.7	2.42	135	23.6	31.8	61	40	23.3	83.0	8th " "
	25.5	2.03	126	22.3	28.1	99	85	31.9	94.3	12th " "
	19.2	1.28	150	20.9	31.3	99	88	29.7	86.5	15th " " sacrificed
C 339	46.6	7.46	63	34.0	21.5	2	0	13.5	81.5	Before treatment
	21.4	3.46	62	32.7	20.2	96	15	17.0	78.4	3rd day "
	24.0	2.29	105	27.6	29.1	79	61	30.3	98.3	6th " "
	25.1	2.16	117	25.0	29.1	95	53	27.5	99.7	8th " "
	21.2	1.94	109	24.2	26.4	98	80	31.2	108.9	10th " " ended
	32.4	3.23	100	25.1	25.2	46	31	23.5	100.8	8th " recovery
	37.9	4.54	84	27.1	22.7	22	15	13.2	88.8	12th " "
	41.4	5.74	72	29.3	21.2	2	0	12.8	—	18th " "
C 337	37.2	5.93	63	33.8	21.2	3	0	9.4	75.9	Before treatment
	14.9	1.51	99	27.7	27.4	82	39	25.5	91.2	9th day "
	18.5	1.65	112	26.9	30.2	97	74	29.2	100.8	13th " " ended
	29.8	2.85	105	25.9	27.1	45	29	20.0	94.6	3rd " recovery
C 323	42.4	7.05	60	34.4	20.7	2	0	15.2	80.2	Before treatment
	20.4	3.50	58	36.4	21.2	86	16	14.3	75.8	3rd day "
	17.4	1.25	139	23.9	33.1	99	84	33.5	102.4	6th " "
C 322	19.3	1.50	129	22.3	28.7	72	—	31.4	101.4	9th day treatment
	23.1	1.85	125	23.3	29.1	79	—	35.8	105.1	10th " " ended
	27.9	2.86	98	26.0	25.4	42	—	20.8	78.2	4th " recovery
	41.9	6.70	63	32.3	20.2	2	—	14.0	79.6	12th " "

course of injections and the period of recovery. Small blood samples for determinations of the reticulocyte count, total red cell count, and hemoglobin content were taken daily during the period of injections, but less frequently after the injections were stopped. By the fourth day a high percentage of the erythrocytes appeared to contain reticulum, and many crenated cells were seen. At first the reticulum was very fine and in some instances difficult to distinguish from crenation. During this time the mean cell volume was little if any increased. Around the sixth to eighth day, nearly 100% of the erythrocytes showed heavy reticulation, differing markedly from that found on the fourth day, and a high mean cell volume was attained, sometimes exceeding twice the initial normal value. In some rabbits it was possible to maintain close to 100% reticulocytosis for more than a week by repeated injections of small doses of phenylhydrazine.

Results and Discussion. In Table I are cited data on the distribution of the acid-soluble P, changes in dimensions and hemoglobin content, and the percentage of reticulocytes in the bloods of several rabbits following the administration of phenylhydrazine. It can be seen that the volume of the packed cells decreased in each animal to ½ or less of the normal value within 3 days after the start of injections, but that further change during longer periods of treatment was slight. The red cell count on the other hand continued to decrease while phenylhydrazine was administered. The mean cell volume (MCV) of the cells seemed to increase in proportion to the number of heavily reticulated cells, reaching values of 150 μ^3, about 2.5 times the normal value. These large cells contain somewhat more hemoglobin per cell than mature erythrocytes, but the corpuscular concentration of hemoglobin decreased markedly, to values about ⅔ of normal. The increases of adenosine triphosphate (ATP) in each instance account for a large part of the increased concentration of organic acid-soluble P (OASP) in the cells. The adenosine triphosphate reached a maximum concentration of approximately 30 mg per 100 cc of cells, but did not increase in concentration further despite continued injections of the phenylhydrazine. The

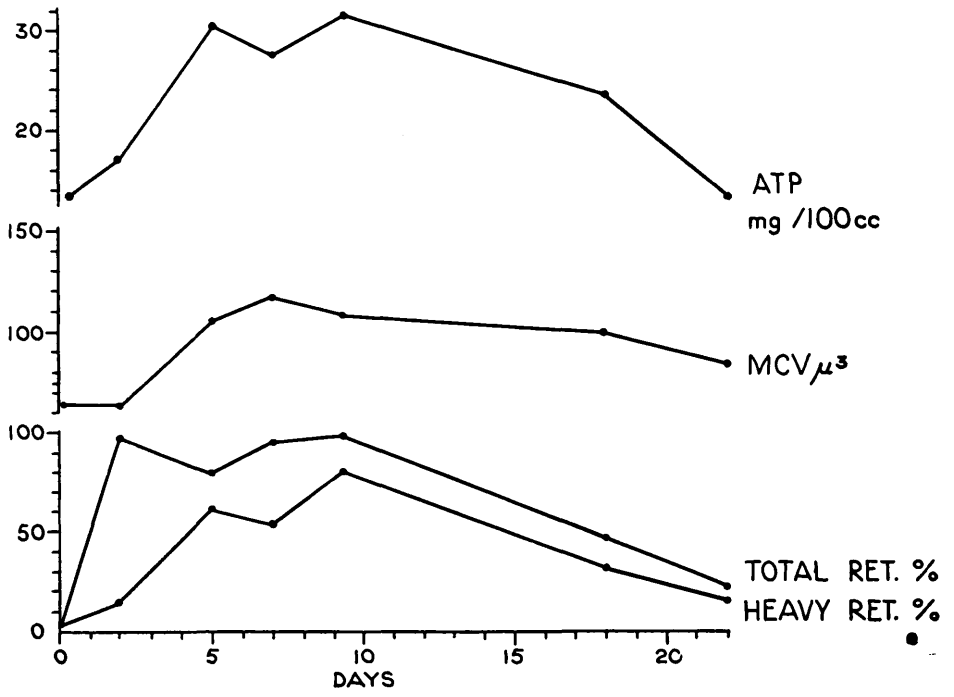

FIG. I RABBIT C 339

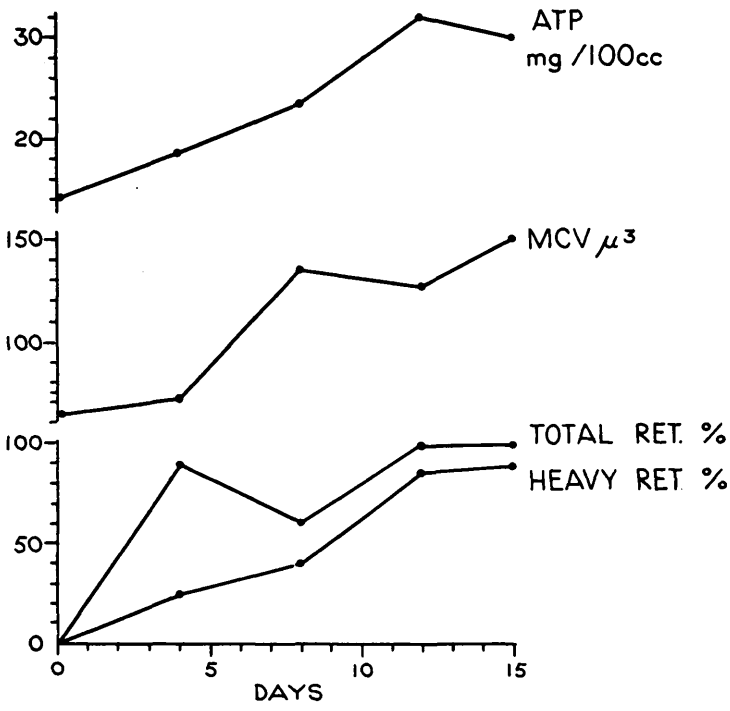

FIG. II RABBIT C 338

concentration of the diphosphoglycerate fraction, determined in some of the samples, did not change significantly.

The charts indicate more clearly than the table a correlation between the changes in the concentration of adenosine triphosphate and the variations in the mean cell size and the proportion of reticulocytes. Two curves portray changes in the reticulocytes, one depicting the proportion of all reticulated cells, the other representing the proportion of coarsely reticulated cells. The curves indicate that the finely reticulated cells are smaller in size than the coarsely reticulated cells, and contain less adenosine triphosphate. In the blood of the animal represented in Chart 1, on the second day after the start of the injections, 96% of the cells were reticulated, most of them finely, but neither the mean cell size nor the adenosine triphosphate content were materially changed; on the ninth day, the proportion of the reticulocytes was about the same as on the second day, but a great majority of the cells showed coarse reticulation and both mean cell size and the adenosine triphosphate content had increased to about twice the initial value. Chart 2 likewise suggests a close relationship between the mean cell size, adenosine triphosphate content, and the number of coarsely reticulated cells. Such evidence suggests the existence of an immature cell, the coarsely reticulated erythrocyte, twice or more the size of the normocyte, containing a high concentration of adenosine triphosphate and a lower concentration of hemoglobin.

The origin and significance of the increased concentration of adenosine triphosphate in the reticulocytes are unknown. Possible relationships between the altered distribution of phosphorus compounds and metabolism of these cells have been discussed earlier.[2]

Summary. Following repeated injections of phenylhydrazine in rabbits, varying degrees of reticulocytosis, up to 100%, occur. With the increasing reticulocytosis the concentration of adenosine triphosphate in the erythrocytes increases from initial concentrations of 10 to 15 mg to 30 mg or more per 100 cc. The mean volume of the cells is greatly increased from around 60 μ^3 up to 150 μ^3, and the corpuscular hemoglobin concentration is decreased from 34 to 22 g per 100 cc of cells.